

College of Saint Benedict and Saint John's University

DigitalCommons@CSB/SJU

Celebrating Scholarship and Creativity Day

Undergraduate Research

4-24-2020

Kombucha's Effect on the Composition of the Human Gut Microbiota, its Connection to Disease, and its Application to Treating an Enteric Strain of Salmonella.

John Hicke

College of Saint Benedict/Saint John's University, JHICKE001@CSBSJU.EDU

Emily Nelson

College of Saint Benedict/Saint John's University, ENELSON001@CSBSJU.EDU

Follow this and additional works at: https://digitalcommons.csbsju.edu/ur_cseday

Recommended Citation

Hicke, John and Nelson, Emily, "Kombucha's Effect on the Composition of the Human Gut Microbiota, its Connection to Disease, and its Application to Treating an Enteric Strain of Salmonella." (2020).

Celebrating Scholarship and Creativity Day. 112.

https://digitalcommons.csbsju.edu/ur_cseday/112

This Paper is brought to you for free and open access by DigitalCommons@CSB/SJU. It has been accepted for inclusion in Celebrating Scholarship and Creativity Day by an authorized administrator of DigitalCommons@CSB/SJU. For more information, please contact digitalcommons@csbsju.edu.

Kombucha's effect on the composition of the gut microbiota, its connection to disease, and its application to treating *Salmonella typhimurium*

John Hicke & Emily Nelson

College of Saint Benedict | Saint John's University



CAPSTONE THESIS

Submitted in partial fulfillment of the requirements for the Integrative Science Major at the College of Saint Benedict | Saint John's University, Minnesota.

April 28, 2020

Abstract

Consumption of kombucha has been shown to promote human gut health and act as an antibacterial agent against enteric pathogens. This thesis investigates if kombucha consumption changes the composition of the human gut microbiota and aims to measure its inhibitory effects on the growth of an enteric pathogen, *Salmonella typhimurium*. We predicted consumption of kombucha will alter the composition of the gut microbiota and demonstrate antibacterial activity in the presence of *S. typhimurium*. Research suggested that alterations in the gut microbiota is associated with multiple chronic diseases. It is further noted that kombucha and other probiotics may be used to restore proper gut health. Based on the literature, we suggest that kombucha has an effect on gut health. To test its antibacterial activity, a Kirby-Bauer Disc Diffusion Assay was performed. Kombucha's zone of inhibition for *S. typhimurium* was compared to two antibiotics (ampicillin, ciprofloxacin) on TSA agar. Both antibiotics displayed consistent inhibitory effects, however, kombucha did not inhibit *S. typhimurium* growth. While kombucha and other probiotics affect the composition of the gut microbiota, kombucha did not demonstrate antibacterial activity against *S. typhimurium*.

Introduction

Fermentation:

Microbial fermentation is a metabolic process performed by microorganisms that convert sugars into acids, alcohol, or gases (Lumen, 2020). This type of metabolic process occurs when microorganisms lack sufficient amounts of inorganic final electron acceptors or do not have genes responsible for fueling the Krebs cycle and electron transport chain (Lumen, 2020). Fermentation readily occurs when microorganisms encounter susceptible substrates that can be used as a fuel source for various metabolic systems (Hasan, Sultan, & Mar-E-Um, 2014). These metabolic interactions within the microorganism's environment include the decomposition of natural materials, which returns chemical elements back to the source, and creates fermented food and beverage products (Hasan, Sultan, & Mar-E-Um, 2014).

Microbial fermentation has been extensively researched and utilized to produce vitamins, alcohol, fermented foods, and fermented beverages (Bisen, 2014). Thus, microbial fermentation provides various benefits to society. For example, fermented foods have demonstrated a multitude of positive effects on the human body and are known to increase food nutritional quality through three main mechanisms. First, fermented foods contain microorganisms that contain anabolic properties allowing it to synthesize vitamins that unfermented food do not have. Secondly, fermentation breaks down indigestible structures, in certain grains and seeds for example, which allows the release of nutritional carbohydrates and proteins. The last mechanism that fermentation is known for is increasing food's nutritional value. Microorganisms have the ability to split indigestible complex sugars into simple sugar, which eases the digestion of foods in the human body (Hasan, Sultan, & Mar-E-Um, 2014). In addition to fermented foods, microbial fermentation can also be applied to the field of medicine by aiding in the creation of antibiotics, such as penicillin and tetracycline (Bisen, 2014).

In terms of fermentation, there are two major types performed by microorganisms: lactic acid fermentation and alcoholic fermentation (Lumen, 2020). Lactic acid fermentation is the chemical conversion of pyruvate and NADH into lactic acid and NAD⁺. This type of fermentation can be used to make fermented vegetables (sauerkraut), sourdough bread, fermented milks (yogurts and cheeses), as well as protein-rich vegetable meat substitutes (National Academy Press, 1992). In comparison, alcoholic fermentation converts sugars to ethanol, carbon dioxide, and other metabolic byproducts. This biological conversion can produce a variety of consumable products such as wine, beer, spirits, vinegar, and kombucha (ScienceDirect, 2017).

Probiotics:

Probiotics are living microbial organisms which when administered in adequate amounts can provide health benefits for the host (Hegazy & Bedewy, 2010). Common foods that contain probiotics are yogurt, fermented/unfermented milk, tempeh, juices, and other beverages like kombucha (Kozyrovska et al., 2012). *Bifidobacteria* and *Lactobacilli* are two probiotics that are generally found in consumable foods and beverages (Hegazy & Bedewy, 2010). Humans tend to consume probiotics because of the extensive health outcomes they have on the internal environment such as aiding in digestion, improving the immune system, and detoxifying the body (Kozyrovska et al., 2012). A commercialized probiotic, *Lactobacillus rhamnosus* HN001, has proven to be beneficial in women's medicine, specifically relating to vaginal and postpartum health (Cheng et al., 2019). Prior research suggests that probiotics can be used to treat a variety of diseases, including liver disease, hypertension, allergies, mental health impairments, and reduce sepsis mortality (Cheng et al., 2019). While there are clear benefits to consuming probiotics in regard to combating certain diseases and promoting overall health, the mechanisms by which probiotics carry out these activities are still of question (Hasan, Sultan, & Mar-E-Um, 2014). Hasan, Sultan, & Mar-E-Um (2014) suggest that probiotics modify the pH of the gut,

stimulate immunomodulatory cells, inhibit pathogens through antibacterial compounds, and outcompete pathogens by binding to their receptor sites. In light of the recent findings, it is critical to investigate the properties of probiotics and how certain probiotics, like kombucha, impacts human health and overall the field of medicine.

Kombucha:

Kombucha is a probiotic beverage that originates from the fermentation of tea leaves leading to the production of a symbiotic microbial community consisting of acetic bacteria and yeasts (Kozyrovska et al., 2012; Kaewkod, Bovonsombut, & Tragoolpua, 2019). While bacteria and yeast live in community in kombucha, it is important to note that they are each responsible for different metabolic pathways that serve the beverage (Kaewkod, Bovonsombut, & Tragoolpua, 2019). Tea leaves undergo fermentation in a sugar-rich environment and yeast cells break down sucrose to glucose (Kaewkod, Bovonsombut, & Tragoolpua, 2019). Ethanol is produced from fermentation, which is a critical step because acetic bacteria relies on ethanol to produce cellulose fiber and organic acids, and this production of acids leads to a drop in the pH value of kombucha during fermentation (Kozyrovska et al., 2012; Kaewkod, Bovonsombut, & Tragoolpua, 2019). These conversions form a cellulose mat, which is formally known as the symbiotic culture of bacteria and yeast (SCOBY). Both ethanol and organic acid products serve to protect the SCOBY by inhibiting other microbes from colonizing (Kozyrovska et al., 2012). The bacteria typically found in probiotics are from the *Lactobacillus* and *Bifidobacterium* genus, while *Saccharomyces boulardii* and *S. cerevisiae* are common yeasts that are stated to be probiotics (Kozyrovska et al., 2012). Kombucha has gathered attention in the scientific community because of the metabolites the SCOBY produces, and its suggested positive health effects (Kozyrovska et al., 2012). The SCOBY's metabolites have been known to promote gastrointestinal health, and more recently it has been documented to demonstrate antimicrobial activity (Kozyrovska et al., 2012). Of interest in this

thesis is how kombucha affects the composition of the human gut microbiota and further exploring its antimicrobial properties against pathogens.

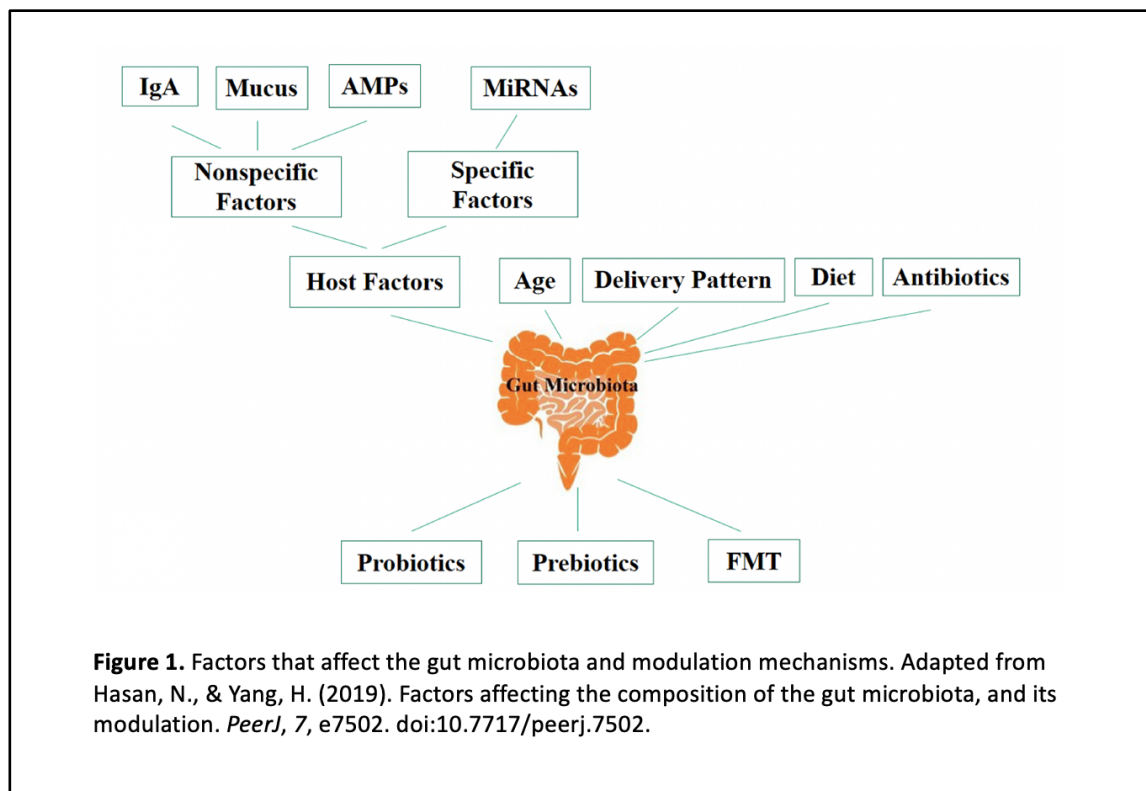
Kombucha and the human gut microbiota:

The term “microbiome” refers to the functional collection of microbes and their genetic material in a particular environmental system, such as the human body (Lu & Liu, 2016). A “microbiota” describes all the microbes in a microhabitat or a specific area of the microbiome. Each human harbors a substantial number of microbial cells, inside and outside of the body. In total, the human microbiome consists of 10 to 100 trillion symbiotic microorganisms (Ursell et al., 2012). When analyzing the human microbiome, most microbial cells are found in the mouth and gut, and on the skin (Lloyd-Price, Abu-Ali, & Huttenhower, 2016). The most sterile areas of the human microbiome are the blood and brain, due to the blood brain barrier, and research notes the lungs may have sterility as well (Lloyd-Price, Abu-Ali, & Huttenhower, 2016).

A specific area of the human microbiome, the gut microbiota, plays a critical role in the body's overall health (Kozyrovka et al., 2012; Singh et. al, 2017). It is known that the human gut microbiota has 10^{14} residential microorganisms and 5,000 species of facultative and strict anaerobes that aid in maintaining internal balance for the human host (Kozyrovka et al., 2012; Singh et. al, 2017). These microorganisms include bacteria, viruses, fungi, and protozoa. In the intestinal microbiota, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, and *Proteobacteria*, are the five types of bacteria that are most often identified, with *Firmicutes* and *Bacteroidetes* being the most prevalent (Kozyrovka et al., 2012; Singh et. al, 2017).

The microbial cells in the gut perform a variety of functions for humans. These include synthesizing vitamins, generating metabolic byproducts for energy usage, and promoting immunity (Singh et al., 2017). The gut microbiota also functions to gather energy from our diet, stimulate

epithelial growth, regulate fat storage, and develop the immune system (Kozyrovska et al., 2012). Therefore, it is important to consider the factors that alter the gut microbiota's bacterial makeup because it can alter the overall health and disease state of an individual. A person's diet, antibiotic usage, age, genotype, and other factors (Fig. 1) play a large role in changing the composition of the gut microbiota (Hasan & Yang, 2019; Kozyrovska et al., 2012). Due to the critical role the gut microbiota plays in creating a healthy environment in the human body, it is of interest to study the mechanisms that could potentially alter the gut microbiota. Microbial modifications in the gut have been shown to have both negative and positive impacts, making it an area of research that is important to understand (Singh et al., 2017).



A change in the gut microbiota has been associated with numerous chronic diseases, such as obesity, type 1 diabetes, non-alcoholic fatty liver disease (NAFLD), hypertension, multiple sclerosis, irritable bowel disorder, and gastrointestinal cancers (Jung et al., 2018; Kozyrovska et al., 2012). Additionally, the overgrowth of *Clostridium difficile*, *Proteus spp.*, and *B. fragilis*, which are intestinal

pathogens, can lead to severe functional disorders (Kozyrovska et al., 2012). Prior research demonstrates that kombucha has healing-like properties on the composition of the gut microbiota in mice with NAFLD. The study found that kombucha decreased the bacteria in the mice's gut that contributed to the progression of NAFLD (Jung et al., 2018). Could the consumption of kombucha reduce the progression of other chronic diseases by beneficially altering the gut microbiota? Based on a primary literature review, we hypothesized that kombucha will beneficially alter the composition of the human gut microbiota, and kombucha consumption can be associated with various chronic disease states.

Kombucha's antibacterial properties:

Bacterial infections among the human population is commonly treated with antibiotics, which have the ability to kill (bactericidal) or inhibit (bacteriostatic) pathogen growth. However, many bacteria have developed specific properties that resist the antibiotic's mechanism of action. In the scientific and medical communities, these strains of bacteria are considered antibiotic resistant (Mayo Clinic, 2020). The Mayo Clinic (2020) explains that while many variables contribute to the emergence of antibiotic resistance, high prescription dosages and wrongful prescription of antibiotics are major factors. Because antibiotic resistance has become a prominent issue in medicine, researchers have placed an emphasis on finding alternative antibacterials in hopes to decrease the use of antibiotics. Battacharya et al. (2016) investigated the antibacterial potential of the traditional beverage, kombucha, to fight bacterial infections, which raises the question: can alternative antibacterial products, like kombucha, be consumed as a method to combat the evolving problem of antibiotic resistance?

Before answering this question, it is important to understand what properties of kombucha may aid in its antibacterial activity. Previous research demonstrates that kombucha has antibacterial properties against various enteric pathogens, such as *E. coli*, *Shigella dysenteriae*, *S. typhimurium* and *Vibrio cholera* (Kaewkod, Bovonsombut, & Tragoolpua, 2019). This study concluded that the presence of

organic acids in kombucha was positively correlated with antibacterial activity, and thus contributed to its inhibitory effect on bacterial growth. However, Sreeramulu, Zhu, and Knol (2000) tested kombucha at a neutral pH and found similar antibacterial activity against pathogenic microbes suggesting there are other compounds that contribute to kombucha's inhibitory properties. Bhattacharya et al. (2016) points to kombucha's polyphenolic content as a main contributor to its bactericidal activity rather than organic acids. Polyphenols are chemicals found in plant-based foods and are primarily known for their antioxidant activity (Sign et al., 2017). This study identified catechin and isorhamnetin in its polyphenolic fraction and concluded it has potent effects against enteric pathogens, suggesting polyphenols offer other health benefits (Bhattacharya et al., 2016).

Fermentation time and the type of tea leaves also affect its antibacterial activity. Bhattacharya et al. (2016) fermented kombucha samples for 0, 7, 14 and 21 days before measuring its antibacterial activity against various enteric pathogen strains such as, *E. coli* O157:H7, *Vibrio cholerae* N16961, *Shigella flexneri* 2a 2457T, and *S. typhimurium* NCT 572. Their results state that without fermentation, kombucha had weak inhibitory effects. Kombucha that was allowed to ferment for one week inhibited the growth of all bacteria strains except *S. Typhimurium* (Bhattacharya et al., 2016). However, the samples that fermented for 14 and 21 days demonstrated equal bacteriostatic and bactericidal activity against all strains of bacteria, except *S. Typhimurium* (Bhattacharya et al., 2016). Although *S. Typhimurium* was more susceptible to 21-day fermented kombucha, resulting in a greater zone of inhibition, Bhattacharya et al. (2016) determined the 14-day kombucha had maximum inhibitory activity. Furthermore, Battikh et al. (2012) found that kombucha fermented from green tea had a larger zone of inhibition against *S. Typhimurium* than black tea. However, it is important to preface that a similar study was conducted recently by Kaewkod, Bovonsombut, & Tragoolpua (2019), which concluded that the type of tea leaf, whether it was green, black, or oolong, did not alter kombucha's inhibition against enteric pathogens.

It is clear that further research should be conducted to better understand what properties of kombucha create its antibiotic-like effects. While this question is important, the aim of the current study is to investigate if kombucha can be used in substitute of an antibiotic to treat an enteric strain of *S. typhimurium*. We hypothesized that kombucha would demonstrate antibacterial activity in the presence of *S. typhimurium*, and thus potentially replacing the common treatment of antibiotics.

Methods

Bacteria isolation:

S. typhimurium (ATCC 13311) was used as the enteric pathogen in the current study. *S. typhimurium* was inoculated from a frozen stock culture into sterile trypticase soy broth (TSB) and grown overnight at 37°C. The overnight culture was used to streak for isolation to ensure that a pure culture was being used to create the bacterial lawns of *S. typhimurium*.

Kombucha type and fermentation process:

One bottle of Deane's Kombucha, Strawberry Lemon flavor, was used for the entirety of this experiment. Deane's kombucha undergoes a continuous brew in 30-gallon oak barrels. Once a mature culture is formed, the SCOBY is added to the oak barrel and fermented for seven days. After seven days, 20 gallons is taken out of the original barrel to be infused with whole, organic fruit for three days. The remaining ten gallons are used as the SCOBY for following brews. After the fruit has been infused, fermentation continues in anaerobic conditions for four to seven more days. For purposes of this experiment, total fermentation time was 14 days (Deane's Kombucha, personal communication, Feb. 13, 2020).

Kirby-Bauer disc diffusion assay:

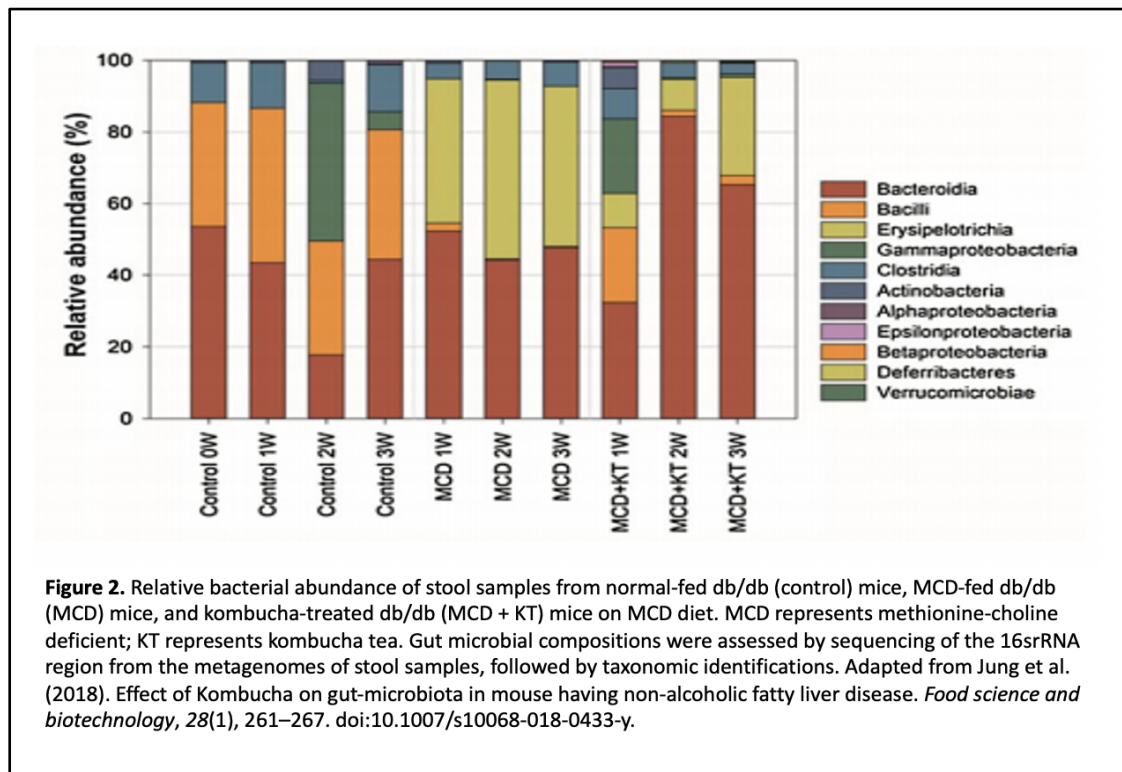
Kirby-Bauer disc diffusion assay was used to test the inhibitory effects of kombucha (Deane's Kombucha: Strawberry Lemon flavor), two antibiotics [Ampicillin (AMP, 10 µg) and Ciprofloxacin (CIP, 5 µg)], and a control. This disc diffusion assay procedure has been previously described by Traub & Leonhard (1994). AMP10 and CIP5 were placed in the center of the bacterial lawn. Whereas experimental discs were soaked in kombucha for two minutes, control discs were soaked in water for two minutes, and were then placed on the bacterial lawn. All plates were incubated at 37°C for 28 hours and zones of inhibition were measured in millimeters (mm). A total of seven trials were used for the experimental groups (kombucha, AMP10, CIP5), and three trials were used for the control.

Statistical analysis:

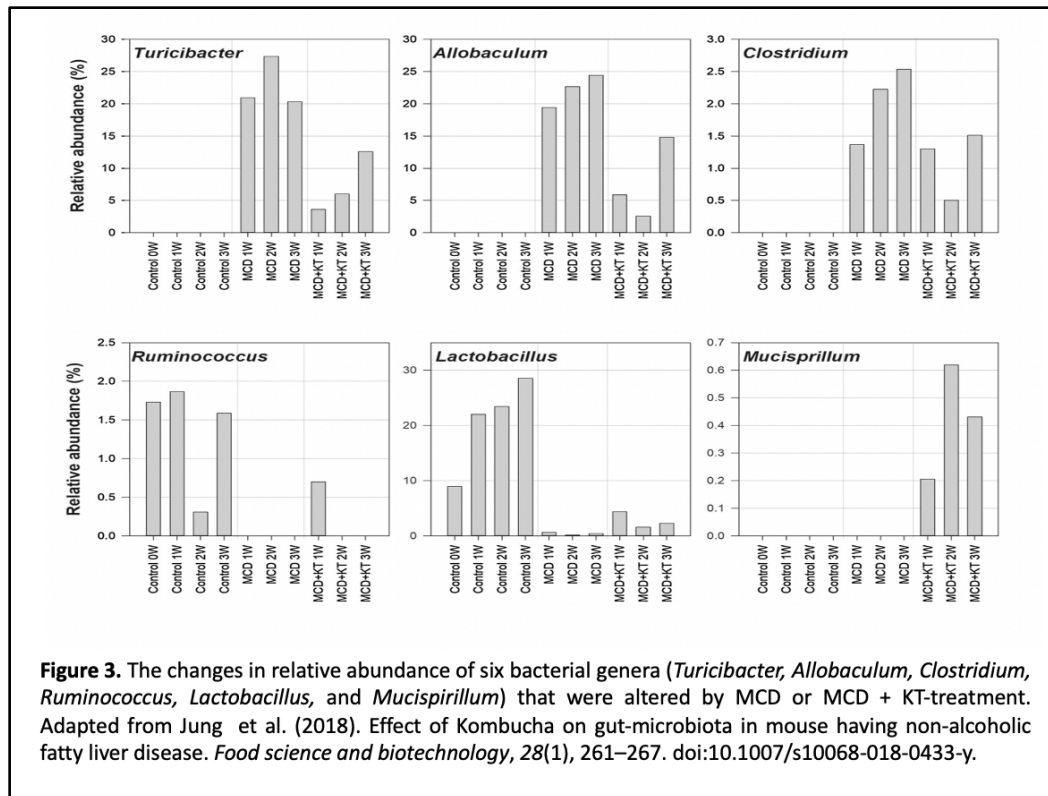
To determine if the inhibitory effects of kombucha, AMP10, and CIP5 were significant, GraphPad Prism Scientific Software was used for statistical analysis (Motulsky et al., 2020). We computed p-values to determine significance using the one-way ANOVA function in GraphPad. Two kinds of comparisons were performed. First, to determine overall significance the mean of all inhibitory zones was analyzed. Following this, we completed a Tukey multiple comparison between kombucha, AMP10, and CIP5 to compare their antibacterial effects against *S. typhimurium*.

Results

Kombucha's effect on the composition of the gut microbiota:



In terms of relative bacterial abundance, MCD-fed (methionine-choline deficient) and MCD+KT (MCD-fed and kombucha tea) experimental groups were associated with an alteration in the composition of the gut microbiota compared to control groups. The *Erysipelotrichi* class (*Firmicute* phylum) were not present in any of the control mice, however, were present in both of the experimental groups. MCD-fed mice had a more significant increase in the relative abundance of the *Erysipelotrichi* class when compared to MCD+KT mice. The *Bacteroidia* class (*Bacteroidetes* phylum) were most abundant in the MCD+KT experimental groups. Furthermore, kombucha paired with the MCD diet led to the highest amount of *Bacteroidia*. Relative abundance of *Bacilli* (*Firmicute* phylum) and *Betaproteobacteria* (*Proteobacteria* phylum) were the highest in control mice, and least abundant in MCD-fed mice (Jung et al., 2018).



There was no significant difference between the dominant genera of the MCD or MCD+KT mice. *Allobaculum* and *Turicibacter* play a role in the pathogenesis of NAFLD and were both significantly decreased in the MCD-fed db/db mice after kombucha tea treatment. Also, the *Clostridium* genus was not present in the control mice. *Clostridium* primarily existed in the MCD-fed mice, and the MCD+KT mice were under-represented in the gut microbiota samples. Bacteria from the *Lactobacillus* genus were more abundant in the MCD+KT mice compared to the MCD-fed mice, however, the control mice represented the highest relative abundance of *Lactobacillus*. Additionally, *Mucispirillum* genus were only found in the MCD+KT mice (Jung et al., 2018).

Kombucha and its application to disease:

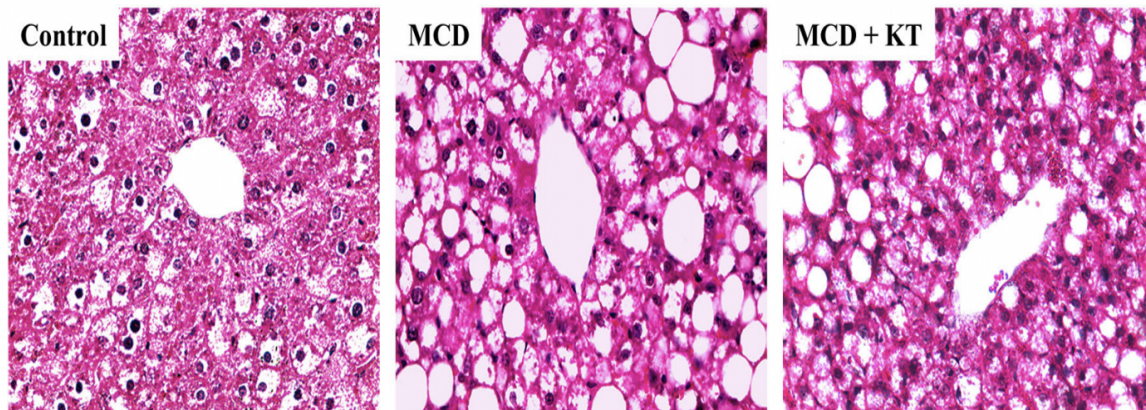


Figure 4. Kombucha reduces fat accumulation in MCD-fed db/db mice. Images represent the histomorphology of the liver sections from normal-fed db/db (control) mice, MCD-fed db/db mice, and MCD + KT-fed db/db mice. Excessive accumulation of macro- and microvesicular fats were observed in livers of MCD-fed mice compared to normal-fed db/db mice and kombucha-treated mice. Adapted from Jung et al. (2018). Effect of Kombucha on gut-microbiota in mouse having non-alcoholic fatty liver disease. *Food science and biotechnology*, 28(1), 261–267. doi:10.1007/s10068-018-0433-y.

Based on histomorphology images of hepatocytes, the consumption of kombucha tea leads to the reduction in fat accumulation surrounding the liver in mice with NAFLD. Image(s) of hepatocytes represent that MCD-fed mice had excessive macrovesicular and microvesicular fat compared to the normal-fed and kombucha-treated mice. Kombucha tea consumption for MCD-fed mice served to display a lipoprotective effect on the liver (Jung et al., 2018).

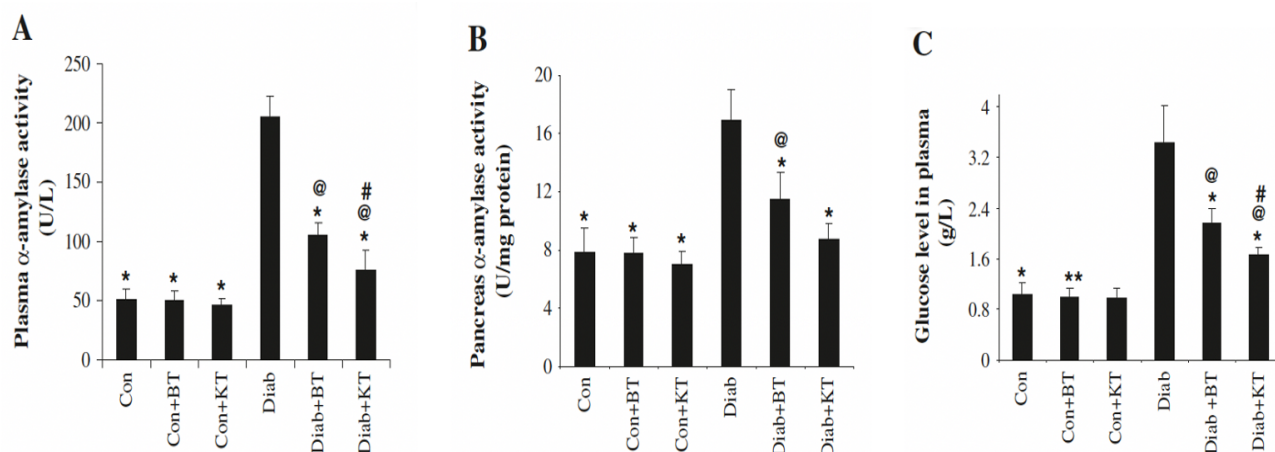
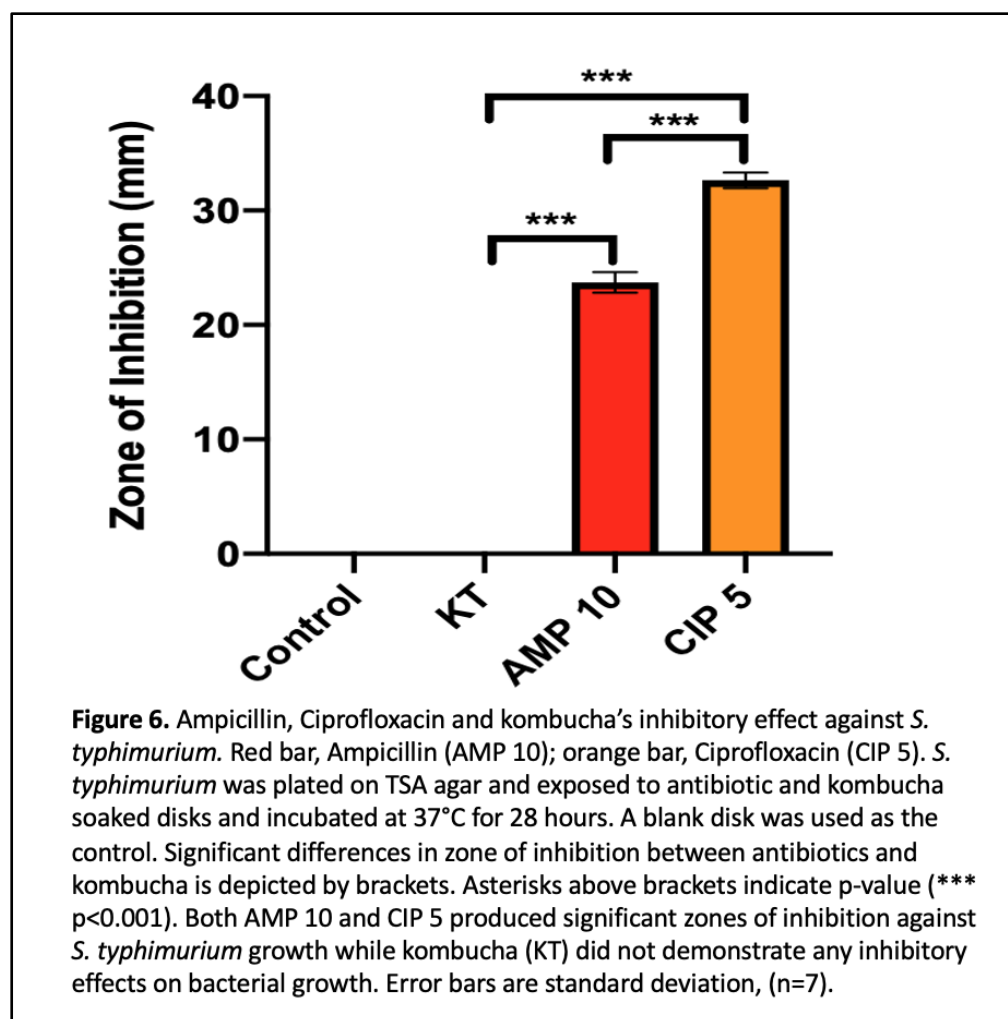


Figure 5. Effect of kombucha tea (KT) and black tea (BT) on α -amylase activity in plasma (A), pancreas (B), and blood glucose levels (C) of surviving diabetic rats. The values are statistically significant and presented as follows: single asterisk (*), $p < 0.05$ vs. control diabetic rats (Diab); commercial at (@), $p < 0.05$ vs. respective control rats (Con); number sign (#), $p < 0.05$ vs. diabetic rats treated with BT (Diab + BT). Adapted from Aloulou et al. (2012). Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. *BMC Complementary and Alternative Medicine*, 12(1). doi: 10.1186/1472-6882-12-63

Kombucha tea and black tea consumption decreases the enzyme activity of α -amylase in the plasma and pancreas and improves elevated blood glucose conditions of diabetic rats. The control group rats endured significant increases of blood glucose concentrations, and α -amylase activities in the plasma [(405 \pm 53%), ($p < 0.05$)] and pancreas [(225 \pm 52%), ($p < 0.05$)]. Consumption of kombucha tea in diabetic rats has a greater inhibitory effect on α -amylase activities in the plasma and pancreas than black tea. Furthermore, kombucha tea significantly suppresses hyperglycemic conditions more effectively compared to black tea. More specifically, kombucha tea supplementation brought about a significant decrease of 50 \pm 11% ($p < 0.05$) when referring to its effect on blood glucose concentration (Aloulou et al., 2012).

Kombucha's antibacterial properties:



Zone of inhibition were measured for the antibiotic and kombucha soaked discs that were placed on the bacterial lawns. The asterisks above the brackets represent the level of p-value significance (***) $p < 0.001$ (Fig. 5). Abundant *S. typhimurium* growth was present on all TSA agar plates after 28 hours of incubation time. An ordinary one-way analysis of variance (ANOVA) results suggested that there was overall statistical significance ($p < 0.001$). Multiple comparisons were used to further analyze the data by separating the antibiotics and kombucha. While both antibiotics, AMP10 and CIP5, demonstrated inhibitory activity against the enteric pathogen, CIP5 had a larger mean zone of inhibition (32.6 mm) which was significantly larger ($p < 0.001$) than AMP10's mean zone of inhibition (23.7 mm).

The kombucha soaked discs did not suppress the growth of *S. typhimurium*, which is represented as a mean zone of inhibition of zero, similar to the control. Both antibiotics had inhibitory zones that were statistically larger than kombucha-treated samples.

Discussion

In the current study, we conducted a literature review on how kombucha affects the gut microbiota and completed an experiment to determine if kombucha's antibacterial properties would demonstrate successful inhibition against *S. typhimurium*. In regard to the gut microbiota, our prediction that kombucha would alter the gut was supported and kombucha consumption can help manage certain chronic diseases. In regard to the laboratory experiment which analyzed kombucha's antibacterial capacity, we originally predicted that kombucha would inhibit the growth of *S. typhimurium* due to its said antibacterial properties. However, this prediction was not supported by the data collected from the Kirby-Bauer disc diffusion assay.

Kombucha's effect on the composition of the gut microbiota and its application to disease:

It is widely known in scientific research that imbalances in the gut microbiota contribute to disease (Hasan & Yang, 2019). Because of this knowledge, many have researched what causes disturbances in the gut in hopes to prevent these agents from generating human disease. Scientific literature has expressed that our diet may be a contributing factor to alterations in gut health (Hasan & Yang, 2019; Singh et al., 2017). Furthermore, Kozyrovska et al. (2012) and Singh et al. (2017) state that probiotic microorganisms help balance the intestinal microbiota. With that being considered, it seems to be known that understanding how diet affects gut health is important because of its association to disease. With that being considered, based on the literature review, it is evident that kombucha affects

the composition of the gut microbiota, and can improve various chronic disease states, such as NAFLD and diabetes (Aloulou et al., 2012; Jung et al., 2018; Kozyrovska et al., 2012).

The study conducted by Jung et al. (2018) demonstrates the beneficial applications that kombucha has against NAFLD. Twelve eight-week old mice were split into three different dietary groups. Four mice received a normal diet and water (control), while the other eight mice consumed a methionine/choline-deficient (MCD) diet. While all eight of the MCD-fed mice drank water, half of the mice were administered 2g/kg of kombucha powder which was dissolved in their water. It is important to mention that MCD diets result in liver injury and are commonly used in research to induce non-alcoholic steatohepatitis (NASH), which is a more progressive form of NAFLD (Machado et al., 2015).

As depicted in Fig. 4, the consumption of kombucha tea led to the reduction in fat accumulation surrounding the liver and serves to produce a lipoprotective effect on the liver (Hyun et al., 2016). The MCD-fed mice without the administration of kombucha had more severe steatosis with increased fat droplets when compared to MCD+KT mice (Jung et al., 2018). Additionally, in relation to the composition of the gut microbiota, Jung et al. (2018) noted that both the control and MCD-fed mice demonstrated a 1:1 ratio of *Firmicutes* to *Bacteroidetes*. However, it is important to mention that patients with NASH have a lower level of *Bacteroidetes*, meanwhile it was found that in MCD+KT mice, *Bacteroidetes* in the intestinal microbiota increased (Fig. 2; Jung et al., 2018). This signifies that kombucha was able to alter the composition of the gut microbiota by increasing the relative abundance of *Bacteroidetes*. This compositional change decreases the 1:1 ratio of *Firmicutes* to *Bacteroidetes*, and thus serves as a protective mechanism against NAFLD. While this is an interesting finding, causal claims cannot be made, and this ratio alteration may only be a correlational finding.

Furthermore, *Allobaculum* and *Turicibacter*, which are dominant bacteria that contribute to the progression of NAFLD, were decreased in the MCD+KT mice (Jung et al., 2018). Kombucha alters the gut

microbiota by lowering the amounts of *Allobaculum* and *Turicibacter* in the gut, as seen in the MCD+KT mice. While this provides additional evidence that kombucha slows the advancement of NAFLD, the mechanism is not understood clearly. It is predicted that the microbial structural or chemical components of kombucha tea might contribute to the decreased progression of NAFLD (Jung et al., 2018).

It is also emphasized that MCD+KT mice had more *Lactobacillus* present in their gut than MCD-fed mice. This is of particular significance because this genus of bacteria has several protective effects that work against the pathogenesis of NAFLD (Jung et al., 2018). Another alteration that occurred in the intestinal microbiota of the MCD+KT mice was a presence of *Mucispirillum* genus (Fig. 3; Jung et al., 2018). Previous studies explain that there is a positive correlation between *Mucispirillum* and the hormone, leptin (Ravussin et al., 2012). This is advantageous because leptin acts on the brain to suppress hunger which will decrease food intake and body fat while increasing metabolic activity (Brennan & Mantzoros, 2006). Although this correlation isn't directly related to NAFLD, the increase in *Mucispirillum* (due to kombucha consumption) may lead to an improved overall health-status and a decrease in body fat.

The consumption of kombucha can also improve hyperglycemia in diabetic subjects. In comparison to black tea, kombucha produces a higher inhibitory effect of α -amylase in the plasma and pancreas of alloxan diabetic rats. Alloxan is a toxin which creates excess reactive oxygen species that damages pancreatic beta cells and inhibits insulin production in response to glucose in the blood (Aloulou et al., 2012). The key digestive enzyme, α -amylase, hydrolyses alpha bonds of starches, which yields maltose and glucose molecules (Aloulou et al., 2012). As the activity of α -amylase increases, blood glucose levels will also increase, and this can lead to hyperglycemic conditions. However, kombucha consumption significantly inhibits the activity of α -amylase, decreasing its ability to break down and

absorb glucose, which prevents the rat from entering hyperglycemic conditions (Fig. 5; Aloulou et al., 2012). While kombucha has a multitude of metabolites that enhance its health benefits, the beverages polyphenolic composition contributes most to its antidiabetic properties of reducing α -amylase activity in the intestines of rats (Aloulou et al., 2012).

Before patients start an antidiabetic drug, life-style interventions are initially suggested in efforts to control blood glucose without the need of any medication (Chaudhury et al., 2017). Since the consumption of kombucha significantly inhibits the activity of α -amylase in alloxan diabetic rats, this type of fermented beverage could be incorporated into a patient's initial life-style intervention plan. The integration of this natural-probiotic beverage early on in one's diabetic diagnosis, may prevent the individual from relying on pharmaceuticals to control their blood glucose levels. This premature action could also avoid the side effects that many diabetic medications have on the individual. Metformin, the best first-line drug therapy for type 2 diabetes, has side effects such as dizziness, muscle pain, difficulty breathing, irregular heartbeat, and others (Nasri & Rafieian-Kopaei, 2014).

Analysis of kombucha's antibacterial properties against *S. typhimurium*:

In the current study, kombucha did not inhibit the growth of *S. typhimurium* which was demonstrated by a zone of inhibition of zero (Fig. 6). However, both CIP5 and AMP10 inhibited the growth of *S. typhimurium* signifying that this strain, derived from *S. typhimurium* (ATCC 13311), was susceptible to these antibiotics. While both antibiotics successfully inhibited growth, CIP5 had a significantly larger mean zone of inhibition (32.6 mm) than AMP10 (23.7 mm), which represents *S. typhimurium* was more sensitive to CIP5 (Fig. 6). This is of interest because *S. typhimurium* has demonstrated antibiotic resistance patterns which has raised concerns and limited treatment options to fight this enteric infection (Wang et al., 2019). Prior evaluations of antibiotic resistance in 11,447 strains

of *S. typhimurium* suggests that *S. typhimurium* is most susceptible to fluoroquinolones, meanwhile, *S. typhimurium* displayed high resistance against ampicillin (Wang et al., 2019). The fluoroquinolone compound, ciprofloxacin, is recommended to treat *Salmonella* infections due to its broad-spectrum properties (Fàbrega & Vila, 2013). These results coincide with our findings as the plated CIP5 antibiotic disc inhibited *S. typhimurium* growth to a greater degree than AMP5 (Fig. 6). However, AMP5 still prevented the growth of *S. typhimurium* suggesting that although its inhibitory effect was not as effective as CIP5, it is not necessarily resistant to *S. typhimurium* (ATCC 13311) in the current study. Understanding which antibiotics have stronger inhibitory mechanisms against *S. typhimurium* is beneficial knowledge for healthcare providers who are looking to treat patients with this enteric infection. If it is understood that ciprofloxacin is a better treatment option than ampicillin for *S. typhimurium* infections, the patient will decrease their exposure to multiple antibiotics, helping to reduce the occurrence of antibiotic-resistance.

It was found that kombucha did not inhibit the growth of *S. typhimurium*, however, this is not supported by previous literature. *S. typhimurium* was found to be sensitive to kombucha at a natural pH (without any pH adjustments), neutral pH of 7, and when kombucha was exposed to heat (Sreeramulu, Zhu, & Knol, 2000). Additionally, it is highlighted that antimicrobial activity increased with the length of fermentation, thus kombucha fermented for a period of 10-14 days, demonstrated maximum zones of inhibition (30-35 mm) against *S. typhimurium* (ATCC 13311) (Sreeramulu, Zhu, & Knol, 2000). Differences in our results may be due to the strain of bacteria used in the present study. The strain used was derived from *S. typhimurium* (ATCC 13311) that Saint John's University has had for many years, so there are likely lab adaptations (mutations). The *S. typhimurium* strain may have mutated and acquired resistant mechanisms that are effective against weaker antibacterial products (kombucha beverage used), which limits our ability to draw conclusions from these results. Furthermore, it is important to recall that Deane's Kombucha carried out its fermentation process in oak barrels, whereas typical kombucha

fermentation is performed in glass brewing vessels (Sreeramulu, Zhu, and Knol, 2000). Future research should explore how the type of fermentation vessel affects kombucha's antibacterial properties. If research finds that one type of fermentative vessel produces greater antibacterial properties, then suggestions could be made in efforts to have consumers receive the highest health-related benefits, and thus be implemented as a useful antibacterial agent. With that being considered, this natural beverage may be used as an alternative to antibiotics, helping to decrease resistance around the globe.

When compared to an antibiotic control, kanamycin, kombucha demonstrated a smaller zone of inhibition than the antibiotic (Bhattacharya et al., 2016). However, it was concluded that kombucha could be used as an antibacterial agent against enteric pathogens due to its polyphenolic content that gives rise to its bactericidal properties (Bhattacharya et al., 2016). While our study does not further support this notion, kombucha's antibacterial properties are still considered cost-effective, accessible, and potentially a safer alternative when compared to the prescription of broad-spectrum antibiotics (Bhattacharya et al., 2016).

Conclusion

Kombucha enhances the composition of the gut microbiota and is applicable to reducing the progression of chronic diseases, more specifically, NAFLD and diabetes mellitus. However, the current study does not demonstrate kombucha's antibacterial properties. Moving forward, kombucha should still be considered as an antibacterial agent against enteric pathogens, such as *S. typhimurium* (ATCC 13311). Based on the literature review and current study, we would recommend individuals to incorporate kombucha into their diet because of its beneficial effects on the gut microbiota and may be helpful to fight enteric infections. Future research should focus on whether or not kombucha has antibiotic-like effects in humans with enteric diseases. If this is established, then kombucha could

potentially be used as a substitute for antibiotics. Furthermore, we would suggest healthcare providers turn to fermented foods and beverages, like kombucha, which contain antibacterial properties as a first line in fighting infections. This would reduce the medical communities' reliance on antibiotics, overall helping to control the spread of antibiotic-resistance.

References

- Aloulou, A., Hamden, K., Elloumi, D., Ali, M. B., Hargafi, K., Jaouadi, B., ... Ammar, E. (2012). Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. *BMC Complementary and Alternative Medicine*, 12(1). doi: 10.1186/1472-6882-12-63
- Battikh, H., Chaieb, K., Bakhrouf, A., & Ammar, E. (2012). Antibacterial And Antifungal Activities Of Black And Green Kombucha Teas. *Journal of Food Biochemistry*, 37(2), 231–236. doi: 10.1111/j.1745-4514.2011.00629.x
- Bhattacharya, D., Bhattacharya, S., Patra, M. M., Chakravorty, S., Sarkar, S., Chakraborty, W., ... Gachhui, R. (2016). Antibacterial Activity of Polyphenolic Fraction of Kombucha Against Enteric Bacterial Pathogens. *Current Microbiology*, 73(6), 885–896. doi: 10.1007/s00284-016-1136-3
- Bisen, P. (2014). The Industrial Uses Of Micro-Organisms. *Microbes in Practice*, (1), 1158–1224. Retrieved from https://www.researchgate.net/publication/299978072_The_Industrial_Uses_Of_Micro-Organisms
- Brennan AM, Mantzoros CS. Drug Insight: the role of leptin in human physiology and pathophysiology—emerging clinical applications. *Nat. Rev. Endocrinol.* 2: 318–327 (2006)
- Deane's Kombucha. (2020, February 13). *Deane's Kombucha Fermentation Process*.
- Cheng, D., Song, J., Xie, M., & Song, D. (2019). The bidirectional relationship between host physiology and microbiota and health benefits of probiotics: A review. *Trends in Food Science & Technology*, 91, 426–435. doi: 10.1016/j.tifs.2019.07.044
- Chaudhury, A., Duvoor, C., Dendi, V. S. R., Kraleti, S., Chada, A., Ravilla, R., ... Mirza, W. (2017). Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. *Frontiers in Endocrinology*, 8. Doi: 10.3389/fendo.2017.00006
- Fàbrega, A., and Vila, J. (2013). Salmonella enterica serovar typhimurium skills to succeed in the host: virulence and regulation. *Clin. Microbiol. Rev.* 26, 308–341. doi: 10.1128/CMR.00066-12
- Hasan, M. N., Sultan, M. Z., & Mar-E-Um, M. (2014). Significance of Fermented Food in Nutrition and Food Science. *Journal of Scientific Research*, 6(2), 373–386. doi: 10.3329/jsr.v6i2.16530
- Hasan, N., & Yang, H. (2019). Factors affecting the composition of the gut microbiota, and its modulation. *PeerJ*, 7, e7502. doi:10.7717/peerj.7502

- Hegazy SK, El-Bedewy MM. Effect of probiotics on pro-inflammatory cytokines and NF- κ B activation in ulcerative colitis. *World J Gastroenterol* 2010; 16(33): 4145-4151. doi: <http://dx.doi.org/10.3748/wjg.v16.i33.4145>
- Hyun, J., Lee, Y., Wang, S., Kim, J., Kim, J., Cha, J., ... Jung, Y. (2016). Kombucha tea prevents obese mice from developing hepatic steatosis and liver damage. *Food Science and Biotechnology*, 25(3), 861–866. doi: 10.1007/s10068-016-0142-3
- Jung, Y., Kim, I., Mannaa, M., Kim, J., Wang, S., Park, I., ... Seo, Y. S. (2018). Effect of Kombucha on gut-microbiota in mouse having non-alcoholic fatty liver disease. *Food science and biotechnology*, 28(1), 261–267. doi:10.1007/s10068-018-0433-y
- Kaewkod, T., Bovonsombut, S., & Tragoolpua, Y. (2019). Efficacy of Kombucha Obtained from Green, Oolong, and Black Teas on Inhibition of Pathogenic Bacteria, Antioxidation, and Toxicity on Colorectal Cancer Cell Line. *Microorganisms*, 7(12), 700. doi: 10.3390/microorganisms7120700
- Kozyrovska, N., Reva, O., Goginyan, V., & Vera, D. (2012). Kombucha microbiome as a probiotic: a view from the perspective of post-genomics and synthetic ecology. *Biopolymers and Cell*, 28(2), 103–113. doi: 10.7124/bc.000034
- Lu, L. J., & Liu, J. (2016). Human Microbiota and Ophthalmic Disease. *The Yale journal of biology and medicine*, 89(3), 325–330.
- Lumen. (2020). Microbial Metabolism: Fermentation, from <https://courses.lumenlearning.com/microbiology/chapter/fermentation/>
- Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine*, 8(1). doi: 10.1186/s13073-016-0307-y
- Machado, M. V., Michelotti, G. A., Xie, G., Almeida Pereira, T., Boursier, J., Bohnic, B., Guy, C. D., & Diehl, A. M. (2015). Mouse models of diet-induced nonalcoholic steatohepatitis reproduce the heterogeneity of the human disease. *PloS one*, 10(5), e0127991. <https://doi.org/10.1371/journal.pone.0127991>
- Mayo Clinic. (2020). Antibiotics: Are you misusing them? Retrieved from: <https://www.mayoclinic.org/healthy-lifestyle/consumer-health/in-depth/antibiotics/art-20045720>
- Motulsky H, Muller JR, Motulsky H, Christopoulos A. GraphPad Prism. Accessed on March 26th, 2020. Available here: <https://www.graphpad.com>.

- Nasri, H., & Rafieian-Kopaei, M. (2014). Metformin: Current knowledge. *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences*, 19(7), 658–664.
- National Academy Press. (1992). *Applications of biotechnology to traditional fermented foods: report of an ad hoc panel of the Board on Science and Technology for International Development*. Washington, D.C.
- Ravussin Y, Koren O, Spor A, LeDuc C, Gutman R, Stombaugh J, Knight R, Ley RE, Leibel RL. Responses of gut microbiota to diet composition and weight loss in lean and obese mice. *Obesity* 20: 738–747 (2012)
- ScienceDirect. (2017). Alcoholic Fermentation. Retrieved from <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/alcoholic-fermentation>
- Singh, R. K., Chang, H.-W., Yan, D., Lee, K. M., Ucmak, D., Wong, K., ... Liao, W. (2017). Influence of diet on the gut microbiome and implications for human health. *Journal of Translational Medicine*, 15(73), 1–17. doi: 10.1186/s12967-017-1175-y
- Sreeramulu, G., Zhu, Y., & Knol, W. (2000). Kombucha Fermentation and Its Antimicrobial Activity. *Journal of Agricultural and Food Chemistry*, 48(6), 2589–2594. doi: 10.1021/jf991333m
- Traub WH, Leonhard B (1994) Agar Disk Diffusion (Bauer-Kirby) Tests with various fastidious and nonfastidious Reference (ATCC) Strains: Comparison of Several Agar Media. *Chemotherapy* 40:374–383.
- Ursell, L. K., Metcalf, J. L., Parfrey, L. W., & Knight, R. (2012). Defining the human microbiome. *Nutrition reviews*, 70 Suppl 1(Suppl 1), S38–S44. <https://doi.org/10.1111/j.1753-4887.2012.00493.x>
- Wang, X., Biswas, S., Paudyal, N., Pan, H., Li, X., Fang, W., & Yue, M. (2019). Antibiotic Resistance in *Salmonella Typhimurium* Isolates Recovered From the Food Chain Through National Antimicrobial Resistance Monitoring System Between 1996 and 2016. *Frontiers in Microbiology*, 10. doi: 10.3389/fmicb.2019.00985